

A MAJOR BREEDING EXPERIMENT WITH TWO ABERRATIONS OF *POLYOMMATUS ICARUS* ROTT., THE COMMON BLUE BUTTERFLY

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A very large-scale breeding experiment with *P. icarus* *abs basielongata* B.&.L. and *antidiscoelongata* B.&.L. has extended over 5 years; the stock is being maintained. From the results of this work the genetic basis of the aberrations can be understood, and it illustrates in a spectacular manner and to an unexpected degree the latent potential for expression in even minor aberrations.

All the breeding work was carried out by L.D. Young and the present analysis is by R.D.G. Barrington.

METHODS

This project has involved carefully controlled and intensive inbreeding of butterfly aberrations on a scale not previously attempted. In order to produce the most extreme aberrations in the shortest time possible three generations have been reared each year, and often several different strains have been maintained simultaneously. The numbers in each brood are often very high (up to 1000 specimens). This maximizes the range of variation within the brood, the most extreme specimens of which are used as parents for the next generation. In this way the expression of the aberrant genes can be developed rapidly.

Such a large concentration of livestock attracts predators such as spiders, earwigs, lacewing larvae and mites, and constant vigilance was needed to keep them under control.

The foodplants used were *Medicago lupulina* L. (black medic) and *Lotus corniculatus* L. (birdsfoot trefoil). Apart from keeping up a constant supply of this food, the trefoil has its own inherent problem as a foodplant due to its reaction to being eaten. It will produce and release hydrogen cyanide gas from the eaten edges of leaves. In fact only a proportion of plants in a population can do this and the numbers vary with locality (Ford, 1975). Whilst *icarus* larvae have an enzyme which breaks down the lethal gas and renders it harmless, which may allow them to feed on the poisonous plants with no ill effects in the field, in captivity high concentrations of larvae feed on individual plants and consequently high levels of the gas are released. It appears that at such a high level the larvae are unable to break it all down and so they will abandon the plant and, for lack of an alternative food-source, eat each other! New plants had to be supplied to replace partially eaten but poisonous plants.

To some extent we avoided the worst effects of inbreeding which are often manifested in weak and deformed adults, low fertility and butterflies that will not pair. Such effects are brought about because rare, harmful recessive genes are brought together into the homozygous state in many specimens where the weakening effects of the gene/s can be expressed. We experienced egg mortality sometimes reaching 50%, but the adults produced were healthy. Bleaching and deformity were rare. The relative health of the stock after so much inbreeding is probably because from each brood many insects are used as parents for the next generation rather than a few or a single pair. Therefore although all the offspring are related they are not necessarily siblings and many of the subsequent pairings will be between insects that

are not brother and sister where the worst effects of inbreeding would normally occur. Outcrossing to wild insects to maintain strength is often done in breeding experiments. We rarely did this mainly because the wild-type genes diluted the strength of variation in the stock and several 'wasted' generations were needed to restore it to its former levels.

RESULTS

Precise details of the early broods are given in an earlier paper (Robertson and Young, 1987). The general pattern of results is given below.

Pure strains

The original ab. *basielongata* female was captured in Hampshire on 19.viii.84 (Fig. 1).

In the first generation from the wild captured aberration approximately 50% of the offspring were wild type and 50% *basielongata*, some of which were more extreme than the parent (Figs 3 and 4). An F_2 was reared from aberrant insects only and produced 100% aberrations but none more extreme than the F_1 examples. An F_2 aberration was paired with a wild, type male and gave an F_1 of 50% aberrations as before. Again aberrations only were paired for the F_2 and in this generation 100% of the emergences were *basielongata*. This stock was then inbred up to the F_8 generation always using the best examples of *basielongata* for pairing, and every brood gave 100% *basielongata*. The later generations produced some extreme forms (Fig. 5 (F_6)). The aberrations *arcuata* Courv., *costajuncta* B.&L., *limbojuncta* Courv. and *basijuncta* B.&L. appeared in the strain in small numbers and in an apparently random manner.

The original ab. *antidiscoelongata* female was captured in Surrey on 18.vi.85 (Fig. 2).

The F_1 generation from the wild aberration contained examples ranging from typical through to minor aberrations (Fig. 6) none as well developed as the aberrant parent. The insects formed a graded series with no clear segregation into type and aberrations. The F_2 showed a similar pattern. These F_2 aberrations were generally not much more developed than those of the F_1 except for one extreme ab. *antiradiata* B.&L. (*Proc. Trans. Br. Ent. Nat. Hist. Soc.* 20:plate III facing p. 43, Fig. 15). The strain was inbred until the F_8 generation. Extreme examples of *antidiscoelongata* began to appear in the F_4 (Fig. 7) and in increasing numbers and in a developed form as the broods progressed (Figs 8 (F_5), 9 (F_5) and 10 (F_5)). The specimen illustrated in Fig. 9 was the only example in the whole breeding experiment to show inward raying of those hindwing spots not connected with the *costajuncta*, *limbojuncta*, *basijuncta* complex. In these later generations the specimens continued to show a wide range of forms from type to extreme aberrations in a continuous series, although type insects decreased and aberrations increased in numbers over the project until the later generations were more or less pure breeding for the *antidiscoelongata* character. As with the *basielongata* stock the four aberrations *arcuata*, *costajuncta*, *limbojuncta* and *basijuncta* appeared randomly.

Crossing the two forms

Good examples of *basielongata* and *discoelongata* were paired to produce an F_1 in May 1987. A proportion of the specimens showed the *basielongata* character and the whole brood was a graded series for *discoelongata* from type to good forms. In general all aberrations, whether of the single or double form, were less well

developed than their respective parents although well developed in comparison with the original wild-caught insects. Inbreeding was continued only with insects showing the double aberration, from which very strong combinations began to occur by the F_3 (Figs 11 and 12). As the inbreeding continued always using the most extreme specimens as parents, the expression of both forms became more extreme. *Basielongata* occurred in 100% of the F_2 (and all subsequent generations) while, as in the pure breeding strain, the *discoelongata* character occurred as a graded series becoming more extreme and more frequent relative to the type form until almost all specimens showed the aberration in some degree in the later generations. Because the *discoelongata* character occurred in no fixed proportions, single and double aberrations continued to occur, both in extreme forms as the broods progressed (Figs 13 (F_6), 14 (F_7), 15 (F_7) and 17 (F_8)). By the second generation of 1989 (the F_8 , which is the last brood covered in this paper) the *discoelongata* character had become so exaggerated in some specimens that it was almost classifiable as *antiradiata* and was not much different from the extreme *antiradiata* that appeared in the F_2 of the pure *antidiscoelongata* strain.

The F_8 , apart from producing some very good combination forms, also contained two exceptional aberrations. One was a very striking male *alba-radiata* B.&L. (Fig. 16) and the other a unique female *basielongata-caeca* B.&L. exhibited at the 1989 Annual Exhibition (*Br. J. Ent. Nat. Hist.* 3: plate III, Fig. 7).

The random appearance of the four minor streaked aberrations continued as well as the occurrence of minor forms of ab. *transversa* B.&L. in which two spots are joined vertically across a vein (Fig. 15). These *transversa* were noted mainly in the later broods.

ANALYSIS

Ab *basielongata* appears to be a dominant gene. The occurrence of a 1:1 ratio of aberrations to type in both F_1 generations from the wild aberration (which may be assumed to have paired with a typical male) and in the F_1 from a bred aberration crossed with a wild, type male strongly suggests that the aberrant parents were heterozygous for the dominant (aberrant) gene. The F_2 generations both showed 100% aberrations. However, problems with poisonous foodplant meant that crippled specimens made scoring for wing patterning difficult, and the small broods were not large enough for the results to be statistically significant. Small broods often show a bias in numbers for type or aberration because there are not enough insects to get a reliable spread of the forms. If the gene for *basielongata* were a pure dominant, one would expect a 3:1 ratio of aberration to type in these F_2 generations but in small broods it is quite possible for the ratio to be biased to give 100% aberrations.

The subsequent broods after the F_2 gave 100% aberrations suggesting a dominant form, but the results of the F_1 generation from the crossing of *basielongata* and *discoelongata* suggest that it is not a pure dominance. The *basielongata* parents for this brood came from the sixth pure-bred generation of the form so one can assume that all specimens were homozygous for the aberrant genes. This being so, all specimens in the F_1 generation from the crossing of the two forms would be heterozygous for the *basielongata* gene, and if the gene were a pure dominant every specimen in the brood should show the characteristic. That some specimens were typical for this characteristic would indicate that *basielongata* is a gene which, although dominant has not achieved complete dominance over the type form so that type characteristics may be expressed in some specimens in the heterozygous state.

The results from ab. *antidiscoelongata* also conform closely to an expected result. Robertson and Young (1987) have previously suggested that it is a simple recessive.

Further examination of the specimens and the results of broods achieved since their conclusions were drawn suggest that the situation is somewhat more complex. It appears to be an example of polygenic (or multifactorial) control. Instead of being controlled by a single mutant gene, the aberration involves several such genes which can work together showing an additive effect. Each gene involved acts like a simple aberrant gene being dominant or recessive to the type form, but there are several of them and they may be a mixture of dominants and recessives (Ford, 1936; Kettlewell, 1973).

With polygenic control all the genes will be in the heterozygous state in an F_1 , so that only those genes that are dominant will exert their effect, but it will not be a complete effect as the recessive genes will not be expressed. The F_1 will consist of a graded series from type through to aberrations no more extreme than the parent, usually less so. The F_2 will show a similar pattern but with some of the recessive genes in the homozygous state as well as some homozygous dominants, so more extreme aberrations may occur. In both generations it would be difficult to give ratios of aberrations and type insects because of the continuous nature of the series. In subsequent generations using only the best aberrations for pairing more and more of the genes would come together in the homozygous state in the same insects and so more extreme aberrations would be produced and the proportion of type insects would be reduced, eventually to zero. This matches very closely the results obtained in breeding from *discoelognata*.

Polygenic inheritance is one of the most common modes of expression of variation and occurs in a wide range of butterfly aberrations such as *Lysandra coridon* Poda (chalkhill blue) ab. *marginata* B. & L. (in which the black border of the upperside of the male insect spreads across the wings) and *Pyronia tithonus* (hedge brown) L. ab. *excessa* Leeds (with extra forewing spots), both of which have been bred by R.C. Revels.

The range of expression of genes

The series of broods has illustrated the variation in expression of the genes. Although a gene may be a simple recessive or dominant and may have a clear-cut effect on the wing pattern, the range of its effect may vary considerably. A very good example of this is *Papilio machaon* L. (swallowtail) ab. *obscura* Frohawk; a heavily melanic form totally dusted with black scaling. This was bred many years ago by L.H. Newman and proved to be a simple recessive. However the melanic specimens that were reared in the F_2 were very variable. Some were dusted with black with the underlying pattern showing through clearly. Others were covered in thick black scaling with virtually no pattern visible. At first glance they appear to be different forms, although they are just different expressions of the same gene (Newman, 1960).

It is possible to select so that, although all aberrations in a brood are controlled by the same gene/s only the most extreme are used for breeding (ie selecting the genes that are being expressed most fully). A strain can then be built up in which the gene is always in an extreme state of expression. One of the earliest and clearest demonstrations of this in Lepidoptera was with *Angeronia prunaria* L. (orange moth). In the type form the adult is of a uniformly orange colour. Ab. *corylaria* Thunb. has the orange restricted to a central band on all four wings by dark scaling. The aberration is controlled by a single dominant, but highly variable gene. By selective breeding, strains can be produced in either direction — with an excessively wide orange band or with the orange restricted to a spot on each wing. The two extremes appear quite different but are under the control of the same dominant gene (Williams, 1946). This situation is possible partly because there is variability in the effectiveness of individual genes and partly because gene expression is often

modified by other genes (modifiers), so selective breeding will work on both the individual gene and on its modifiers to produce a combination that allows the aberrant gene to be expressed most fully.

The present experiment has done just this. With the *discoelongata* character, for example, in the later generations most specimens would probably have all had the aberrant genes in a homozygous state so they are all being expressed. However the strain is still progressing, with more and more extreme aberrations appearing. In the early broods selection would mainly have been for specimens with the most genes in a homozygous state. In the later broods, when all genes were homozygous, selection would have been for the most extreme genes. The most developed aberrations that have appeared in this series of experiments bear little resemblance to the original parents, but they are under the same genetic control.

Crossing the two forms showed how this selection had worked. When the initial cross was made between good *basielongata* and *discoelongata* each aberration would probably have had type genes for the other aberration. Effectively each aberration was being paired to a type insect (for that character). However in the F_1 from that cross, aberrations occurred that were more strongly expressed than the aberrations in the original F_1 generations from the wild aberrations although they were effectively the same thing (in those original F_1 specimens and in the F_1 specimens from the crossed forms all the aberrant genes would be in a heterozygous state). This happened because since the original F_1 generations from the wild aberrations selection had occurred for those genes that were most strongly expressed, and while none of the recessive genes would have been expressed in any F_1 generation, the dominant genes were expressed and they had been 'strengthened' by the time the F_1 from the cross of the aberrations occurred.

The only comparable experiment to the present one on a British butterfly was done by P.M. Sheppard (Ford, 1945) in which starting with a minor aberration, he inbred the darkest examples of *Euphydryas aurinia* Rott. (marsh fritillary) in his broods for 14 years. Towards the end of the experiment some extremely dark forms were emerging (ab. *bicolor* Wehrti). An example of this aberration is very well illustrated by A.D.A. Russwurm (Howarth, 1973). More extreme forms than the illustrated example were bred but by this time inbreeding was beginning to take a serious toll and deformities were common. As with *icarus* ab. *discoelongata*, *bicolor* was under polygenic control.

The present experiment has now gone beyond 14 generations and involves two aberrations. It will be of great interest to see just how far the markings can be developed in further generations.

The radiata forms

As previously mentioned two extreme *radiata* forms have appeared in the stock. The female *antiradiata* from the F_2 of the pure bred *discoelongata* is clearly a very extreme expression of *discoelongata* with the spots being exceptionally large and streaked, and it is now being approached by specimens reared in the most recent broods. That it occurred so early in the experiment must be put down to the fact that a chance combination of genes in the polygenic system occurred which allowed for extreme expression of the *discoelongata* character. Inbreeding now means that this chance combination is occurring with some regularity.

The *alba-radiata* from the F_8 of the crossed forms may be something different. Certainly no similar hindwing variation has appeared before in these experiments. White ground-colour often goes with extreme spotting forms and may be part of the expression of *radiata* in some specimens. It may well be a spontaneous mutation

which can occur when a strain is maintained and inbred for many generations. This occurred in a culture of *Pieris brassicae* L. (large white) maintained by B.O.C. Gardiner (Gardiner, 1962). After about 80 generations the abs *coerulea* Gardiner and *albinensis* Gardiner suddenly appeared. The former has a pale blue ground colour, and the latter is albinistic (see Russwurm, 1978). Both were simple recessives. Alternatively it could be the most extreme expression of the *discoelongata* series, the hindwing character only appearing with a specific gene combination. With luck further breeding from this strain will prove the point one way or the other.

The inward rayed hindwing form from the F₅ of the pure-bred *discoelongata* series bears no relation to any other aberrations in the experiment and is most likely to be a spontaneous mutation.

Other aberrations in the stock

A bilateral gynandromorph was bred in the F₅ generation of the crossed forms (*Br. J. Ent. Nat. Hist.* 2: plate IV, facing p. 48, Fig. 2). It is caused by imperfect gene separation in the first cellular division in the egg. This is a chance phenomenon and is not related to the rest of the experiment.

The aberrations in the *arcuata* complex (including ab. *arcuata*, *costajuncta*, *limbojuncta* and *basijuncta*) appeared randomly in the experiment. Nothing firm can be said about their genetics, although three of them (excluding *limbojuncta*) seem to be connected in some way. R. Barrington has bred all three in an F₁ from a *basijuncta* female and L. Young has had similar results (Robertson & Young, 1984). They are probably not connected to the *basielongata* and *discoelongata* forms, but being common enough forms in the field, they may have 'been in the genes' of the captured female aberrations or the males with which they had paired. *Limbojuncta* is a much rarer form in the field and rarely occurs by itself, more often occurring on specimens showing extreme fore or hindwing forms. It is less likely that it happened to be in both wild stocks. It may have some connection with the *basijuncta* and *discoelongata* forms. Robertson & Young (1987) suggest an environmental influence on the occurrence of the *arcuata-costajuncta-basijuncta* complex in combination with a genetic basis. This may well be so, but the whole complex requires more work before it can be understood.

A single female ab. *caeca* occurred in the F₈ of the crossed forms, no sign of spot obsolescence having been seen before in the strain. It is not a rare form in *L. coridon* and *L. bellargus* Rott. (adonis blue) but is more so in *icarus*. In the former two species it is almost invariably associated with a weakness manifested in 'shot-holed' or crumpled wings. I have not seen enough of the form in *icarus* to make a similar generalization, although the present specimen and a male of the form taken by R. Barrington in Eire in 1984 were quite perfect. The *icarus* example here may have occurred as the result of a weakness in the strain caused by inbreeding. But as it is perfectly formed and of normal size, it is more likely that it was due to a spontaneous mutation.

Ab. *transversa* is a form usually associated with a weakness which causes venational defects. The veins appear to act as boundaries for individual spot markings so any vein reduction will allow spots to join vertically down the wing. Only minor forms involving two spots occurred, probably due to a low level of inbreeding weakness.

CONCLUSIONS

The experiment has demonstrated the inheritance of the aberrations *basielongata*

